EVIDENCE FOR A TRANSIENT DIGESTIVE
TRACT IN VESTIMENTIFERA

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Abstract. — A medial process has been observed at the base of the branchial plume of juvenile vestimentiferans; this structure persists for only a short period in early developmental stages of the worms. In the youngest specimens observed, there is a continuity from the distal, ciliated opening of the process to a posterior basal opening. This complete apparatus appears to be the mouth-gut-anus of the Vestimentifera, heretofore not observed. The ciliated opening (mouth) of the process connects with a ciliated duct (esophagus/foregut) which, in turn, leads to the presumptive trophosome (midgut), then into a spacious, thin-walled region (hindgut) and, finally, opens to the exterior via a posterior, terminal anus. In later stages the midgut is filled with presumptive endosymbiotic bacteria; its communication with the hindgut is lost, and the anal opening disappears. In still later stages the medial process, with its oral opening, also is lost and the endosymbionts of the midgut are isolated internally in tissue that will become the trophosome; the foregut persists in later stages, even in adults, as a rudimentary strand of tissue traversing the brain. We suggest that free-living bacteria pass through the mouth of early juveniles to the midgut where they come to lodge in epithelial cells, the bacteriocytes of the future trophosome. We further suggest that the side of the worm with the medial process, the nerve trunks and a prominent vestimental ciliated field is ventral, as has been suggested previously; the side with the excretory and genital pores and the pulsatile blood vessel (heart) is dorsal. Such an orientation, coupled with the aspect of the septa and medial mesentery of the opisthosome, emphasizes more clearly the close relationship of the phylum Vestimentifera to the phylum Annelida.

Bacteria have been demonstrated in the trophosome of vestimentiferans (Cavanaugh et al. 1981, Cavanaugh 1983b) and pogonophorans (Southward et al. 1981, Southward 1982), in the ctenidia of certain bivalves (Cavanaugh 1983b) and in association with the body wall of certain marine nematodes (Ott et al. 1982), turbellarians (Ott et al. 1982) and oligochaetes (Giere 1981). These and other studies have suggested a chemoautotrophic role for these bacteria that provides or augments the nutrition of their hosts (Cavanaugh et al. 1981; Felbeck et al. 1981, 1983; Southward et al. 1981; Cavanaugh 1983b; Felbeck 1983;

Fisher & Hand 1984; Dando et al. 1985). Among numerous ultrastructural and biochemical studies of the relationships of these symbionts with their hosts, however, there are few suggestions as to the source of the bacteria or how they gain access to their ultimate location in the body of the host (for extensive bibliography see Jones & Bright 1985).

It has been suggested: (1) that a possible endocytosis of bacteria into bivalve epithelia is followed by phagocytosis into vacuoles (Southward 1986); (2) that bacteria of pogonophores are probably derived from free-living species in muds of the sea bottom
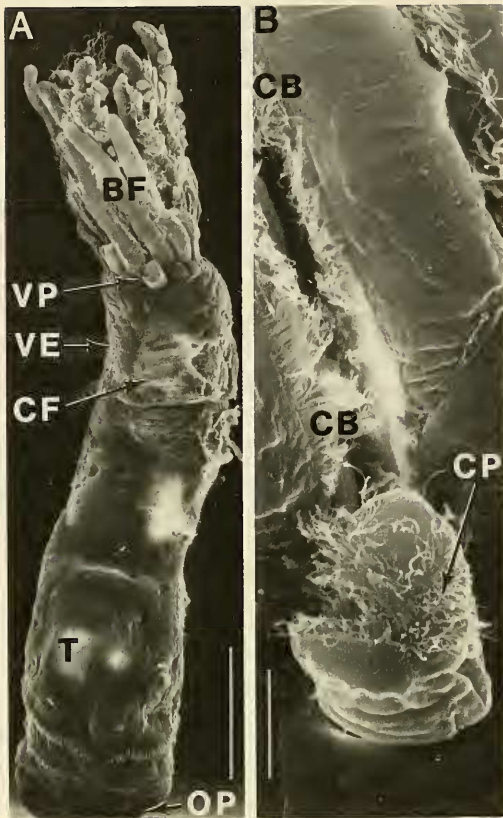
Fig. 1. *Riftia pachyptila* Jones, juvenile, SEM; *Alvin* Dive 984, Rose Garden, Galapagos Rift, 2451 m depth. A, Ventral view, showing medial process (VP) at base of branchial filaments (BF); B, Same, enlargement of medial process. Scale bars: A = 200 μm; B = 20 μm; CB, cilia of branchial filaments; CF, ciliated field; CP, cilia of process; OP, opisthosome; T, trunk; VE, vestimentum.

(Flügel & Langhof 1983); and (3) that, in the case of *Solemya reidi* Bernard, eggs of adult females are infected by bacteria. These bacteria develop in the tests of brooded larvae and following ingestion of test fragments, the larval gut degenerates at metamorphosis and releases bacteria into the hemocoel, whence they are presumed to pass to the ctenidia [this sequence of events is suggested in a dissertation by Gustafson (Reid & Brand 1986)]. Bacteria have not been observed in developing eggs of *Riftia pachyptila* (SLG, personal observations using TEM) or in developing sperm (Jones & Gardiner 1985, Gardiner & Jones 1985), suggesting that some method other than a direct parental inoculation may be operative.

In worms of the phylum Vestimentifera (Jones 1985) there is a transient access to the interior of juveniles (and larvae?) (Jones, in press). In *Riftia pachyptila* Jones, *Oasisia alvinae* Jones and juvenile vestimentiferans from Axial Seamount, Juan de Fuca Ridge (probably *Ridgeia piscesae* Jones), a medial process arises at the boundary of the obturacular (branchial) and vestimental regions on the so-called neural side (Figs. 1, 2, VP; for methods for scanning microscopy see Jones & Gardiner 1985). The process is present on specimens of *R. pachyptila* up to at least 15.5 mm total length. In larger specimens (for example, 111 mm total length) the process has disappeared.

In small juveniles of *R. pachyptila, O. alvinae* and ?*R. piscesae* the medial process possesses a ciliated aperture that leads to an internal ciliated duct; this traverses the brain and excretory organ, passes posteriorly through the vestimentum between the two major longitudinal blood vessels (Fig. 3D) and, at least in *R. pachyptila,* communicates with the trophosome via paired structures noted as trophosomal chambers (Jones, in press). In larger specimens the duct has atrophied such that its course through the excretory organ to the trophosome cannot be followed. Jones (in press) suggested that free-living bacteria from the microenvironment of the larvae/juveniles are taken up, at random, by the medial process and are transported to the presumptive trophosomal tissue via the ciliated duct; here the bacteria are acquired by putative bacteriocytes, perhaps by phagocytosis. Once associated with the internal milieu of the young *Riftia,* those bacteria that can survive do so as endosymbionts.

Examination by transmission electron microscopy (TEM; for methods see Gardiner & Jones 1985) reveals that the luminal
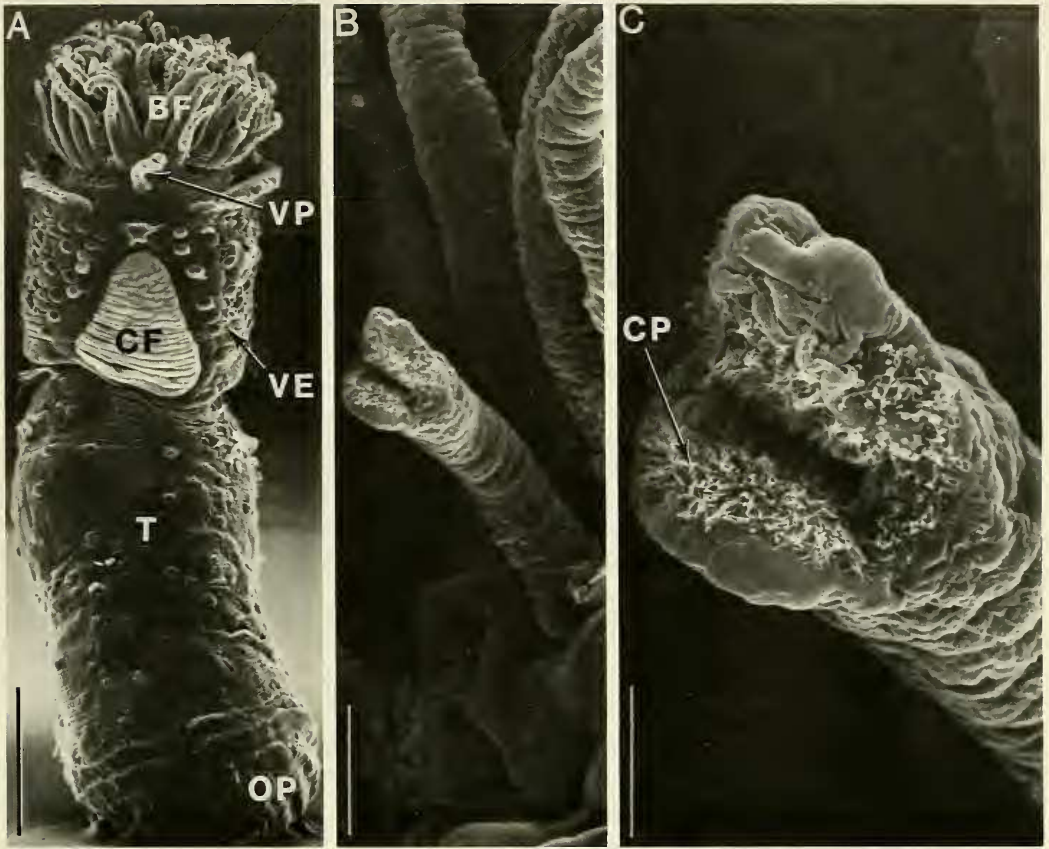
Fig. 2. ?*Ridgeia piscesae* Jones, juvenile, SEM; *Alvin* Dive 1413, Axial Seamount, Juan de Fuca Ridge, 1546 m depth. A, Ventral view, showing medial process (VP) at base of branchial filaments (BF); B, Same, enlargement of distal end of medial process; C, Same, further enlargement of medial process, showing cilia surrounding opening leading to ciliated duct. Scale bars: A = 400 μm; B = 50 μm; C = 20 μm; CF, ciliated field; CP, cilia of process; OP, opisthosome; T, trunk; VE, vestimentum.

wall of the duct in the anterior region of the vestimentum comprises at least three or four multiciliated cells about 4–5 μm in thickness (Fig. 4A). The lumen of the duct, which is obscured by cilia, is about 5–7 μm in diameter. A thin basement lamina separates the luminal wall from an outer wall that consists of approximately 10 longitudinal muscle cells (Fig. 4A, single arrow). A second basement lamina is situated between the layer of muscle cells and the epithelium of the body wall (Fig. 4A, double arrows). In the region of the brain, the duct appears somewhat triangular in shape when viewed in cross-section (Fig. 4B). However, it displays the same organization as anteriorly, except that approximately 15 muscle cells occur in the outer layer. The cells of the luminal wall display a complex pattern of interdigitation that prevents an accurate estimate of the number of cells present (Fig. 4C). As the duct passes posteriorly from the brain to the excretory organ, the outer muscular wall loses its integrity. Only the inner luminal wall remains (approximately 3.5–12 μm thick) surrounding the ciliated lumen (about 7 $\times$ 20 μm), which, in the present case, is compressed frontally (Fig. 4D).

In the course of its traverse of the muscles and connective tissue of the vestimentum,

the lumen of the duct undergoes variation in its dimensions (8 × 22 μm, 8 × 11 μm, 4 × 9 μm), as does the thickness of its wall (1–10 μm, 4.5–5.5 μm, 3–19 μm). This variation is probably an artifact of fixation, based on the compression and asymmetry of the duct lumen and duct wall. In the region of the anterior extension of the trunk and trophosome onto the vestimentum, the duct is situated between the ventral blood vessel and the well-muscularized dorsal blood vessel (Fig. 3, VV, DV). The lumen and wall of the duct appear irregular in shape. It has not yet been possible to trace the duct into the trunk region using TEM.

The bulk of the trophosome of *R. pachyptila,* and, by extension, that of other vestimentiferan species, consists of endosymbiotic bacteria in bacteriocytes (Fig. 5A). Only blood vessels and their associated muscle cells, the peritoneum of the trophosomal lobules, and the cell membranes and cytoplasm of the bacteriocytes that house the endosymbionts, are structures of the vestimentiferan host (Cavanaugh et al. 1981; Jones 1981a, b, 1984; Cavanaugh 1983a; Bosch & Grassé 1984a, b; de Burgh 1986). Plasma membranes of adjacent bacteriocytes are shown by TEM observations to have a junctional complex characteristic of those invertebrate epithelia that line outer surfaces and luminal surfaces such as gut, gonoducts, etc. (Green & Bergquist 1982, Lane et al. 1987). This complex consists of a belt desmosome (*zonula adhaerentes*) and an accompanying region of septate junction (Fig. 5B, C). The occurrence of such a junctional complex between bacteriocytes, coupled with the probability that bacteria are transported to the presumptive trophosomal tissue via a ciliated duct with an an-

terior external opening is suggestive of a heretofore unobserved characteristic of the Vestimentifera, that, during early development a temporary mouth and gut are present.

Early juvenile stages of vestimentiferans from the Juan de Fuca Ridge have been observed in which trophosomal bacteria are present, as well as still earlier stages in which trophosomal bacteria have not yet been acquired; in these latter stages a complete gut can be demonstrated (see below).

The distal end of the medial process is provided with a ciliated vestibule that is continuous with an internal ciliated duct passing posteriorly through the brain (Fig. 6A). Based on the extension of the external cuticular covering as a lining for the vestibule and its abrupt proximal termination (MLJ, pers. observ.), we propose that the vestibule represents the stomodeum of the developing worm and thus its mouth. Internal to the cuticular termination, epithelial cells with staining properties different from those of the vestibular epithelium, line a ciliated duct (Fig. 6B). This layer of cells, at this stage about 10 μm thick with a lumen diameter of about 12 μm, may be the beginning of the foregut (Fig. 7C, FG). The foregut passes through the brain and connects to the midgut (Fig. 7C, MG). The midgut, with a lumen diameter of about 4 μm at this stage, has a layer, from 6 to 8 μm thick, of dark-staining epithelial cells; these cells have fewer cilia than those of the foregut. The midgut leads to a spacious, thin-walled hindgut (Fig. 7C, HG), which opens externally through a posterior, terminal anus (Fig. 7A–E, A).

In later stages of development the midgut accumulates bacteria, presumably within the

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Fig. 3. *Oasisia alvinae* Jones, juvenile, TEM; *Alvin* Dive 1221, Clam Acres, 21°N on East Pacific Rise, 2618 m depth. Montage of cross-section in posterior region of vestimentum, showing ciliated duct (D) between ventral blood vessel (VV) and muscularized dorsal blood vessel (DV). Scale bar: 10 μm; B, bacteria in trophosomal lobule; P, peritoneal cell of trophosome.
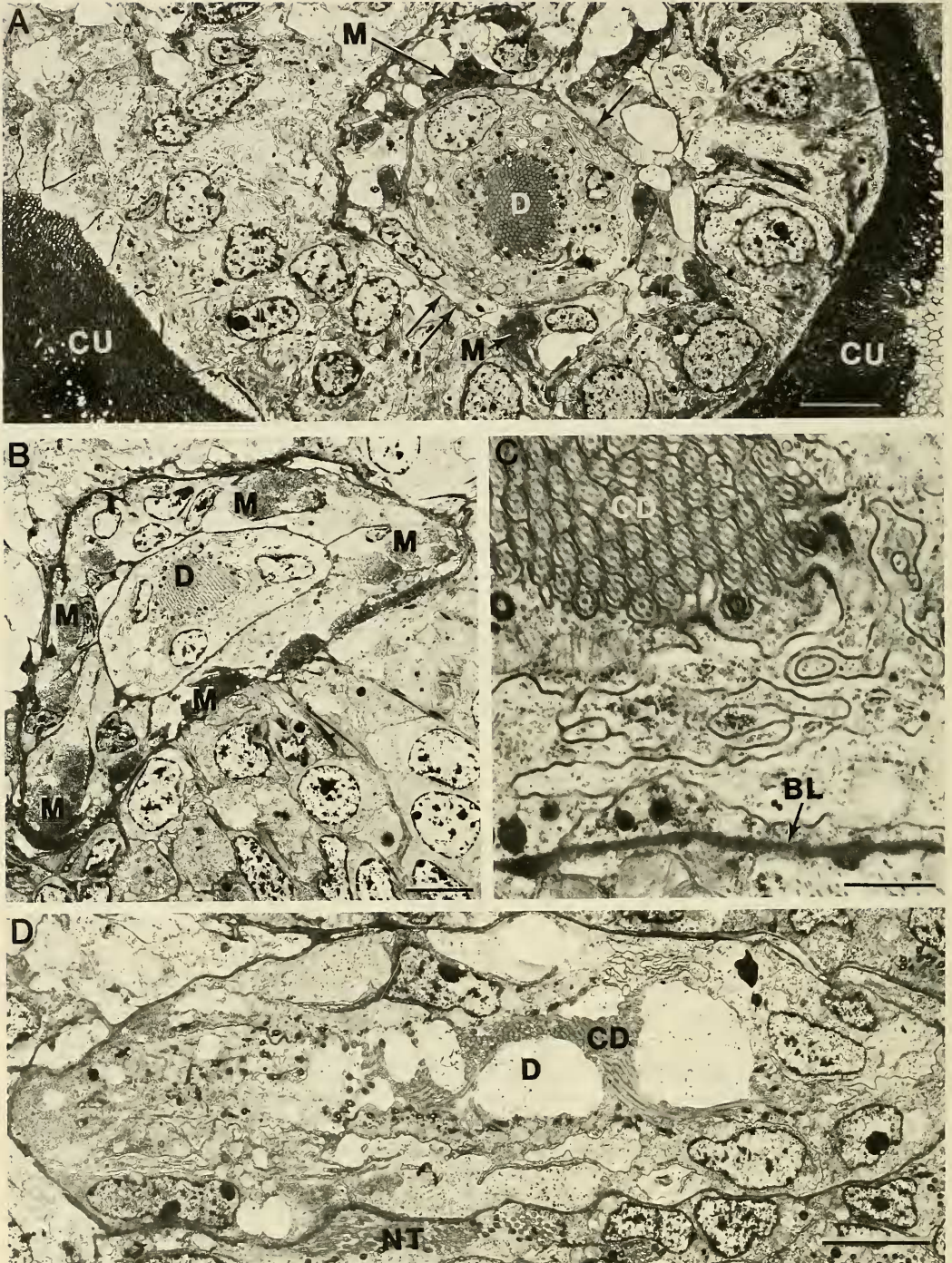
Fig. 4. *Oasisia alvinae* Jones, juvenile, TEM; *Alvin* Dive 1221, Clam Acres, 21°N on East Pacific Rise, 2618 m depth. A, Duct (D) in anterior region, showing basement lamina (single arrow) surrounding ciliated cells of
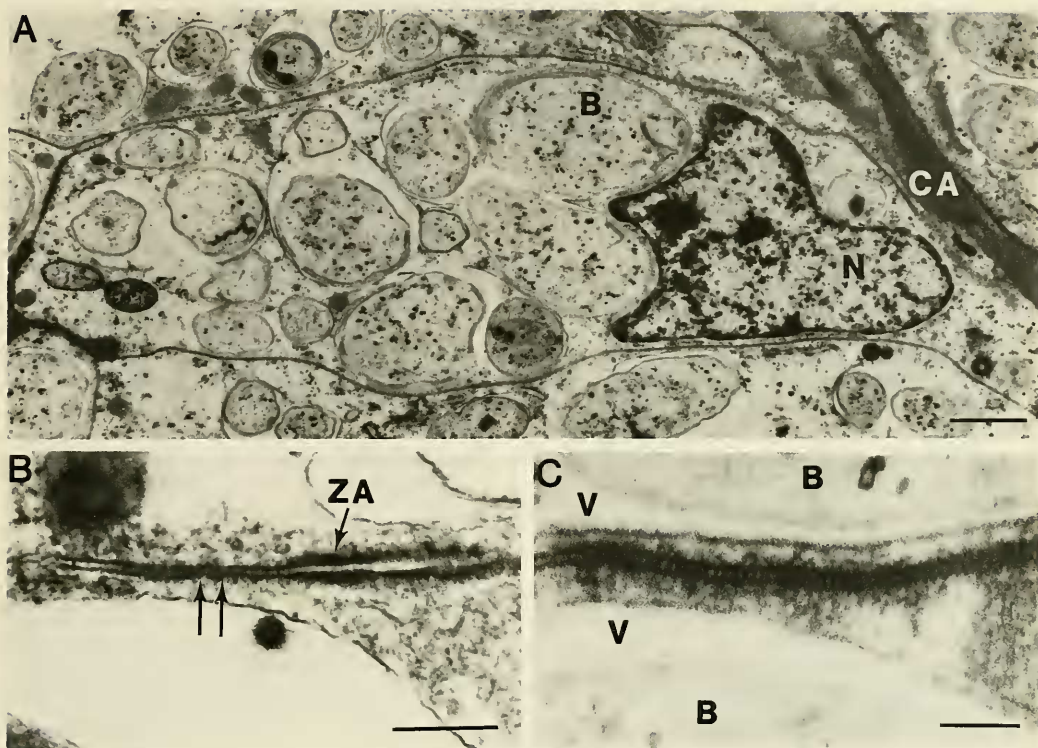
Fig. 5. *Riftia pachyptila* Jones, adults, TEM, bacteriocytes in trophosome; A, C: *Alvin* Dive 1221, Clam Acres, 21°N on East Pacific Rise, 2618 m depth; B: *Alvin* Dive 990, Rose Garden, Galapagos Rift, 2451 m depth. A, Bacteriocyte adjacent to capillary (CA). Note amount of space in bacteriocyte occupied by bacteria (B). B, Junctional complex between bacteriocytes, showing *zonula adhaerentes* (ZA) and region of septate junction (double arrows). C, Enlargement of portion of septate junction. Scale bars: A = 1 μm; B = 0.3 μm; C = 0.1 μm; N, nucleus of bacteriocyte; V, vacuole containing bacteria.

epithelial cells (Fig. 7B, TR); these cells are the original bacteriocytes of the presumptive trophosome. We postulate that the bacteria are incorporated into the bacteriocytes by phagocytosis. The later development from this original state to the well-vascularized, lobular condition of the adult trophosome is not known nor is the status of the "trophosomal chambers" found in somewhat older juveniles of *Riftia pachyptila* (Jones, in press).

The presence of a gut in juvenile vestimentiferans is anatomically and phylogenetically significant. In the past, there has

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luminal wall and second basement lamina (double arrows) separating layer of longitudinal muscle cells (M) from epithelium of body wall. B, Duct at level of brain. C, Enlargement of ciliated cells of luminal wall of duct, showing interdigitation of plasma membranes. D, Slightly oblique cross-section of duct in region of excretory organ (NT). Scale bars: A, B, D = 5 μm; C = 1 μm; BL, basement lamina; CD, cilia of duct; CU, cuticle of body wall.
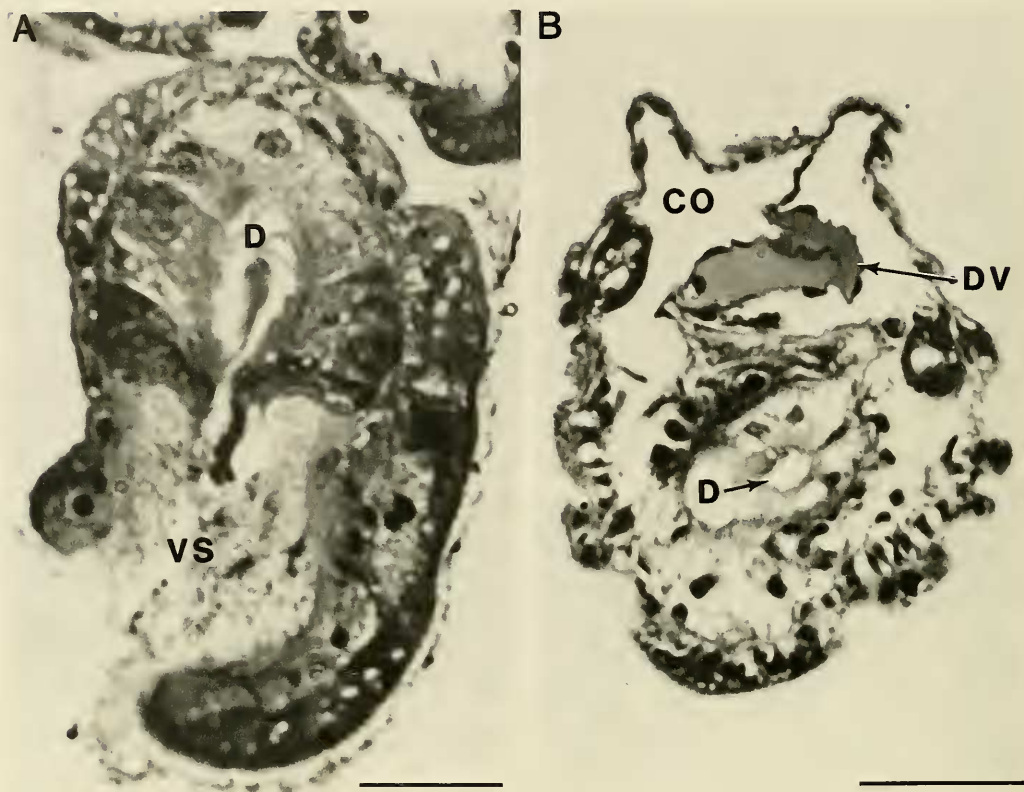
Fig. 6.   ?*Ridgeia piscesae* Jones, juvenile, light micrographs of semi-thin sections (1.5 μm thick); *Alvin* Dive 1413, Axial Seamount, Juan de Fuca Ridge, 1546 m depth. A, Oblique cross-section through anterior end, showing ciliated duct (D); B, Cross-section through trunk region, showing dorsal vessel (DV) and ciliated duct (D). Scale bars: A = 20 μm; B = 30 μm; CO, coelom; VS, vestibule.

been controversy concerning the dorsal-ventral orientation of the pogonophorans and vestimentiferans (Jones 1981a). The opinion of one of us (MLJ) that "dorsal" and "ventral" of Vestimentifera (and Pogonophora) were defined by the location of a pulsatile, well-muscled blood vessel (heart) and of the nerve cord, respectively, was based on the assumption of a close affinity to the Annelida (Jones 1981a). The present observations strengthen this assumption; that is, the ciliated opening (mouth) of the medial process leads to a ciliated duct (esophagus/foregut) that passes through the brain (supraesophageal and subesophageal ganglia and commissures) and leads to the trophosome (midgut). In later stages this passage atrophies to a thin strand of tissue. This arrangement supports annelidan affinities and suggests a possible homology between bacteriocytes and the gut epithelium of a common ancestor to the Annelida and Vestimentifera. Further, the side of the animal with the medial process, the nerve cord and the extensive ciliated field of the vestimentum is ventral. Because a similar morphological arrangement has not yet been observed in the Pogonophora, their affinities and orientation remain unclear; however, we do feel that the phyla Vestimentifera and
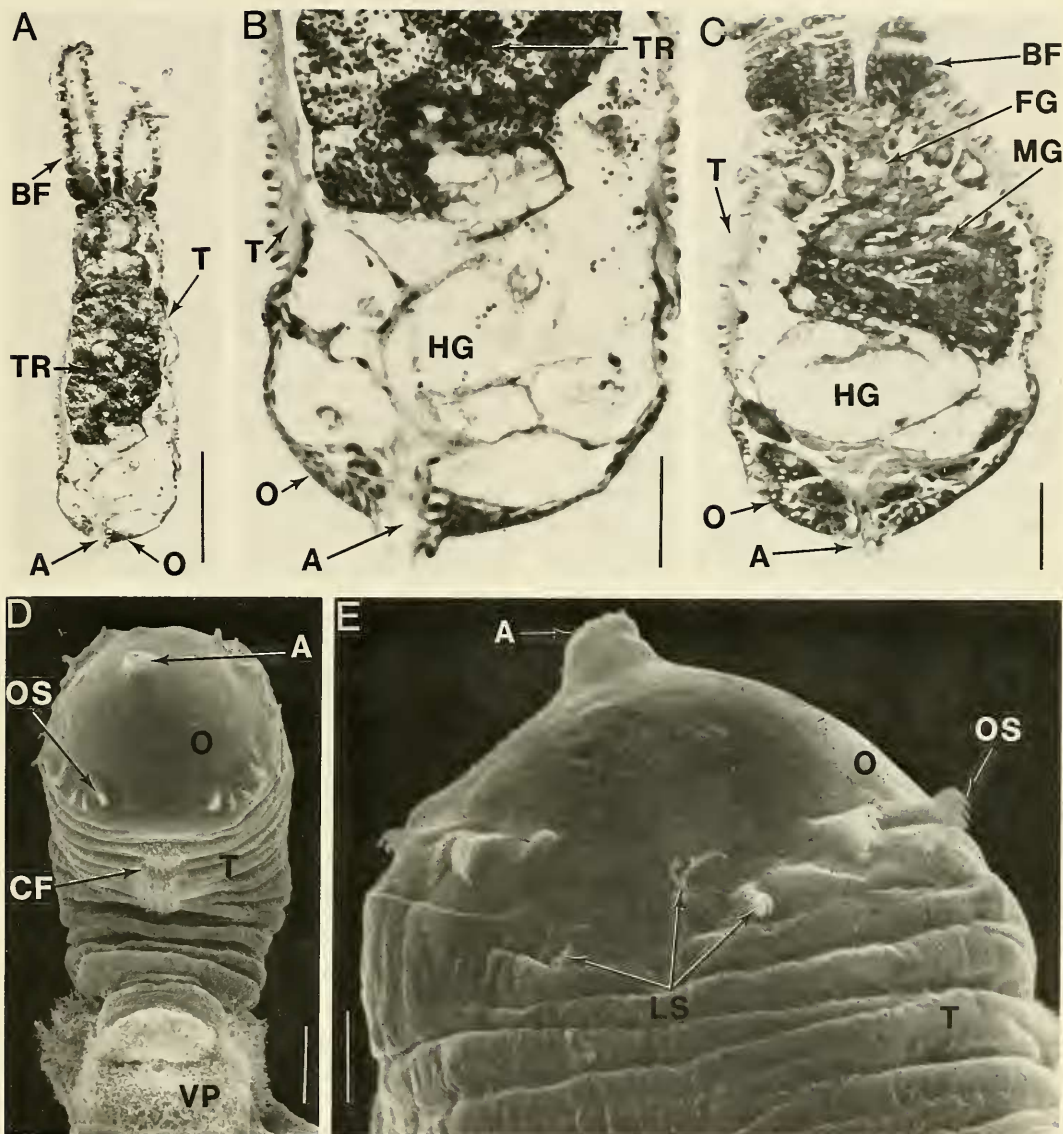
Fig. 7.   ?*Ridgeia piscesae* Jones, juveniles, A–C, light micrographs of semi-thin sections (2 μm thick), D–E, scanning electron micrographs; *Alvin* Dive 1413, Axial Seamount, Juan de Fuca Ridge, 1546 m depth. A, Frontal section, showing internal organization; B, Same, enlargement of posterior region, showing presumptive trophosome (TR), hindgut (HG) and anus (A); C, Frontal section of second specimen, lacking trophosomal bacteria, showing regions of foregut (FG), midgut (MG) and hindgut (HG) and anal opening (A); D, Specimen mounted with anterior end down, ventral view; Same, lateral view of left side of opisthosomal region. Scale bars: A = 100 μm; B = 50 μm; C, D = 25 μm; E = 10 μm; BF, branchial filament; CF, ciliated field; LS, larval setae; T, trunk; O, opisthosome; OS, opisthosomal setae; VP, medial process.

Pogonophora are relatively closely related to each other and both are related to the phylum Annelida.

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